Published in final edited form as:

Expert Opin Ther Pat. 2014 December ; 24(12): 1383–1395. doi:10.1517/13543776.2014.977865.

Triggering receptor expressed on myeloid cells (TREM) receptor family modulators: a patent review

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Abstract

Introduction—Triggering receptor expressed on myeloid cells (TREM) receptors and TREM-like transcript (TLT; or TREML) receptors of the immunoglobulin superfamily are known as key modulators of host immune responses. TREM-1 (CD354) and TREM-2 share the transmembrane adaptor DNAX-activation protein of 12 kDa (DAP12), but they possess separate stimulatory and inhibitory functional roles, especially in myeloid cells.

Areas covered—This review covers findings related to TREMs and TLTs published in patent applications from their discovery in 2000 to the present. New roles for TREM-1, TREM-2, TLT-1 and TLT-2 in maladies ranging from acute and chronic inflammatory disorders to cardiovascular diseases and cancers are discussed. Putative endogenous ligands and novel synthetic peptide blockers are also discussed.

Expert opinion—So far, therapeutic use of activators/blockers specific for TREMs and TLTs has been limited to pre-clinical animal models. TREM-1 is an immediate therapeutic target for acute and chronic inflammatory conditions, especially sepsis. Certain mutations in DAP12 and TREM-2 manifest into a disorder named polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOS), and newly identified TREM-2 variants confer a significant increase in risk of developing Alzheimer's disease. This makes TREM-2 an attractive therapeutic target for neurodegenerative diseases.

Keywords

CD177; DNAX activation protein of 12 kDa; Heat shock protein 60; High-mobility group box 1; Immunomodulator; Peptidoglycan recognition protein 1; Toll-like receptor; Triggering receptor expressed on myeloid cells; TREM-like transcript

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Disclaimer

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Financial & competing interests disclosure

Devendra K Agrawal was supported by grants from NIH (USA) (ROIHL104516, ROIHL112597, ROIHL116042, ROIHL120659). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

1. Introduction

The cDNAs encoding TREM-1 and TREM-2 receptors were first identified by comparison to a homologous natural killer cell receptor, NKp44 (1, 2). Subsequently, the genes that encode TREMs, TLTs and NKp44 mapped together next to the major histocompatibility complex on human chromosome arm 6p21.1 (3). TREM-1 and TREM-2 are both transmembrane glycoproteins (~26–30 kDa) and bear a single extracellular immunoglobulin domain (see Figure 1). The transmembrane domains of TREM-1 and TREM-2 couple to the transmembrane domain of the adaptor protein DAP12 (DNAX-activation protein of 12 kDa; also known as killer cell activating receptor-associated protein, tyrosine kinase binding protein)(4).

Recruitment of DAP12 to TREM-1 and TREM-2 is characterized by positive-negative charge attraction at the transmembrane level (5, 6). DAP12 is also an adaptor protein for various activating receptors and inhibitory receptors belonging to the related CD300 and Fc Receptor families (5, 7). DAP12 exists as a disulphide-linked homodimer and engages with tyrosine kinases (i.e. Src and Syk) through its cytoplasmic immunoreceptor tyrosine-based activation motif (ITAM) (5, 6).

2. Lessons from genetic mouse models

2.1 TREM-1 deficiency

Genetic deletion of the *TREM-1* gene in *TREM-1*^{-/-} mice actually mitigated the inflammatory response and disease severity following viral infection with influenza virus or parasitic infection with *Leishmania major* (8). TREM-1-deficient mice and control wild-type mice showed equal ability to clear the pathogens (8). In another study, however, *TREM-1/3*^{-/-} mice experienced increased mortality after intratracheal infection with *Pseudomonas aeruginosa* (9). This phenotype was characterized by poor neutrophil recruitment and impaired bacterial clearance in bronchoalveolar lavage fluid (9). Lastly, *TREM-1*^{-/-} mice were less susceptible to diethylnitrosamine-induced hepatocellular carcinogenesis than control wild-type mice, reflecting a possible pro-oncogenic role of TREM-1 (10).

2.2 TREM-2 deficiency

TREM-2^{-/-} mice experienced improved survival and enhanced bacterial clearance following infection with *Streptococcus pneumoniae* when compared to control wild-type mice (11). Moreover, macrophages isolated from TREM-2-deficient mice displayed greater induction of cytokines (i.e. IL-6 and TNF-α) by the TLR ligands LPS, CpG or zymosan, compared to wild-type macrophages (12). Based on these results, TREM-2 function exerts anti-inflammatory effects. Kang and colleagues generated a transgenic line of mice over-expressing TREM-2 under control of CMV promoter (13). The tumor-associated antigen GA733-2 was down-regulated in multiple tissues from the transgenic mice, including Peyer's patches and lamina propria lymphocytes (13). Over-expression of TREM-2 also decreased both proliferation and migration of MC-38 cells (mouse colon adenocarcinoma cell line) *in vitro* (13).

2.3 TLT-1 deficiency

TREM-like transcript 1 (TLT-1) receptor is confined to platelets and megakaryocytes (14). Platelets isolated from *TLT-1^{-/-}* mice displayed poor aggregation *in vitro* (15). Accordingly, *TLT-1^{-/-}* mice exhibited extended tail-bleeding times and increased mortality following challenge with LPS or the cecal ligation and puncture model of polymicrobial sepsis (15, 16). The above results from genetic mouse models of TREM-1, TREM-2 or TLT-1 deficiency highlight the involvement of these receptors in immune responses and in cancer.

3. TREM-1 functions

3.1 Membrane-bound TREM-1 signaling

TREM-1 possesses the ability to amplify signaling by the Toll like receptors (TLR) TLR4 or TLR2, which recognize a wide range of bacterial, fungal, viral and self components (17, 18). In particular, co-stimulation of neutrophils or monocytes with the TLR4 ligand lipopolysaccharide (LPS; endotoxin) and an anti-TREM-1 agonistic monoclonal antibody produced a synergistic increase in pro-inflammatory cytokine expression (17, 18). Intriguingly, TREM-1 and TLR4 have been shown to transport to and co-localize within lipid rafts on the surface of neutrophils upon treatment with LPS or anti-TREM-1 agonistic antibody (19). TLR4 signaling is transmitted through the adaptor protein myeloid differentiation factor 88 (MyD88) and subsequent activation of nuclear factor κ B (NF- κ B) and mitogen-activated protein kinase pathways. While TREM-1 can amplify MyD88 signaling, TREM-2 can diminish MyD88-dependent TLR4 signaling, as demonstrated in macrophages (12, 20).

It is desirable to develop quantitative assays for markers of TREM-1 activation as a tool to screen and measure the efficacy of putative TREM-1 inhibitors or activators. Here we provide a brief survey of TREM-1 downstream signaling pathways. In monocytes, treatment with an anti-TREM-1 agonistic antibody stimulated intracellular Ca^{2+} mobilization and tyrosine phosphorylation of extracellular signal-related kinase 1/2 and phospholipase C- γ (1). Microarray analysis revealed that treatment of monocytes with an anti-TREM-1 agonistic antibody or LPS increased the production of cytokines including IL-1 β , IL-6, IL-8, TNF- α , MCP-1 and GM-CSF (21). Uniquely, TREM-1 activation, as opposed to LPS, induced monocyte expression of M-CSF and osteopontin (or secreted phosphoprotein 1) (21, 22). At the same time, it was evident that TREM-1 activation attenuated LPS-mediated induction of certain IL-12 family subunits in monocytes (21). Treatment of neutrophils with anti-TREM-1 agonistic antibody was shown to initiate degranulation and release of myeloperoxidase and nitric oxide (17).

3.2 TREM-1 expression

In addition to neutrophils, monocytes and macrophages, TREM-1 is also expressed on non-myeloid cell types including epithelial and endothelial cells (23–25). Elevated TREM-1 levels are indicative of acute and chronic conditions including sepsis, pneumonia, inflammatory bowel disease and rheumatoid arthritis (26–28). TREM-1 itself is up-regulated by components of fungi, Gram-negative and Gram-positive bacteria, LPS and CpG-oligonucleotides (17, 29). *TREM-1* gene transcription is positively regulated through AP-1,

cAMP, NF- κ B, Vitamin D receptor and hypoxia response elements, and negatively regulated through a PU.1 element within the *TREM-1* promoter (25, 30–33).

3.3 Soluble TREM-1

Human TREM-1 is a 234 amino acid type I transmembrane protein composed of a signal peptide, an extracellular domain of 184 amino acids, a transmembrane domain and a short cytoplasmic domain of only 5 amino acids (see Figure 1). Crystal structure data are inconclusive as to whether or not TREM-1 forms homodimers (34, 35). The TREM-1 extracellular domain can also be found as a soluble form called soluble TREM-1 (sTREM-1). It is proposed that sTREM-1 may arise directly from a splice variant or following the shedding of membrane-bound TREM-1 by metalloproteinase-mediated proteolytic cleavage (36, 37).

sTREM-1 has proven to be a valuable diagnostic and prognostic marker since it is easily detected using immunochemical assays on biological fluid samples (37–39). For example, sTREM-1 levels are elevated in sera from patients with sepsis and in bronchoalveolar lavage fluid from patients with bacterial or fungal pneumonia (26, 27, 37).

4. TREM-1 ligands and peptide blockers

4.1 TREM-1 decoy receptors

We have learned from numerous studies that blocking TREM-1 signaling can mitigate hyper-activation of immune responses. Current efforts are aimed at more precise methods of inhibiting TREM-1 activation using small molecules and peptide mimetics. sTREM-1 is proposed to act as an endogenous decoy receptor that binds TREM-1 ligands and prevents their engagement to membrane-bound TREM-1 (40, 41). Soluble receptors have limited use as therapeutic agents since they are subject to a high degree of degradation. Nonetheless, sTREM-1 has been instrumental as the basis for development of short peptides designed to thwart ligands away from the ligand-binding domain of membrane-bound TREM-1.

Gibot and colleagues focused on portions of TREM-1's putative ligand-binding domain that are highly conserved across humans, rats and mice (39, 40). Short peptides referred to as P1 and LP17 (also called P5) were derived from the human or mouse TREM-1 complementarity determining regions 2 and 3, respectively (see Table 1) (39, 41). In a competition experiment, either mouse P1 or LP17 peptide competed away the binding of soluble mouse TREM-1-IgG1 chimera to a ligand of unknown identity on peritoneal exudate cells taken from mice subjected to cecal ligation and puncture (41). Treatment of human monocytes with LP17 *in vitro* attenuated LPS-mediated induction of TNF- α and IL-1 β , confirming the ability of the peptide to block cellular TREM-1 (40). *In vivo* administration of mouse LP17, more so than P1, demonstrated a protective effect against lethality following LPS challenge in mice (39, 41). Further studies have also shown that *in vivo* administration of LP17 improved outcomes in rodent models of sepsis, inflammatory bowel disease, rheumatoid arthritis and cancer (28, 41).

Since LP17 competes for binding with TREM-1 ligands, it is presumed to work as a decoy much like sTREM-1. LP17 is taken from the complementarity determining region 3 that

corresponds to the “F” β strand within the TREM-1 extracellular domain (35, 41). It was postulated that a tyrosine residue in the “F” β strand could mediate TREM-1 homodimerization. LP17 might also act by disrupting this mechanism (35, 41). Peptides based on LP17 sequence with one or more amino acids that are substituted conservatively have also been suggested as putative peptide blockers of TREM-1 (39).

4.2 TREM-1 ligands of unknown identity

For several years following the discovery of TREM-1 in 2000, no endogenous TREM-1 ligands had been identified. A general observation was made that neutralization of TREM-1 with a TREM-1-Fc fusion protein impaired the ability of serum samples taken from patients with sepsis to activate monocytes *in vitro* (42). Similarly, it was shown that an anti-TREM-1 antibody blocked platelet-induced neutrophil activation (43, 44). Also, TREM-1 can bind specifically to platelets from healthy subjects and to neutrophils from septic patients (43, 44). The results suggest the presence of TREM-1 ligands on platelets and neutrophils, although their identities are unknown. Mariani and colleagues developed methods for an immunochemical detection system based on a human TREM-1-IgM fusion protein that bound to TREM-1 ligand(s) of unknown identity on neutrophils isolated from septic patients (44).

4.3 CD177 as a TREM-1 ligand

Knowing the actual identity of TREM-1 ligands greatly increases our vision of TREM-1 function. It also paves the way for inventing synthetic derivatives based on the endogenous ligands that are more potent agonists or antagonists. Here we provide a brief survey of the known TREM-1 ligands (see Figure 2). Colonna and colleagues first identified CD177 (also known as NB1 or PRV-1) as an endogenous TREM-1 ligand (45). This was elucidated by using a cDNA library made from neutrophils isolated from human patients with sepsis. Follow-up studies demonstrated the binding of soluble TREM-1 to HEK293 cells over-expressing CD177(45). Also, anti-CD177 monoclonal antibody competed with binding of TREM-1 ligands to neutrophils from septic patients (45). Interestingly, CD177 is enriched on neutrophils and CD177 alloantibodies are implicated in immune neutropenia and transfusion-related lung injury (46, 47). CD177 was shown also to interact with platelet endothelial cell adhesion molecule-1 via immunoprecipitation and surface plasmon resonance experiments. Additionally, anti-CD177 antibody inhibited transendothelial migration of human neutrophils *in vitro* (47).

4.4 Peptidoglycan recognition protein 1 as a TREM-1 ligand

Stennicke and colleagues later identified Peptidoglycan recognition protein 1 (PGLYRP1; also known as PGRPS, TAG7) as a TREM-1 ligand (48, 49). PGLYRP1 was identified as a TREM-1 binding protein via immunoprecipitation and mass spectroscopy following incubation of soluble TREM-1-Fc protein with peptidoglycan-treated neutrophils and cross-linking (48, 49). PGLYRP1 was capable of inducing TREM-1 activation using a BWZ.36/NFAT-LacZ cell line reporter system (49). Furthermore, anti-PGLYRP1 antibodies attenuated TREM-1 activation upon stimulation with either an agonistic anti-TREM-1 antibody or peptidoglycan-treated neutrophils (48, 49). Mammalian PGLYRP1 is expressed

in polymorphonuclear leukocytes and is freed from granules upon their activation (50). PGLYRP1 contains a peptidoglycan binding domain and possesses bactericidal properties against Gram-positive bacteria more so than Gram-negative bacteria (50). TREM-1 interaction with PGLYRP1 is in alignment with TREM-1 partnering to pattern recognition receptor signaling.

4.5 High-mobility group box 1 as a TREM-1 ligand

Recently, High-mobility group box 1 (HMGB1) was identified as a murine TREM-1 binding protein via immunoprecipitation following incubation of soluble TREM-1-Fc fusion protein with hepatocytes isolated from diethylnitrosamine-treated mice (10). While HMGB1 is naturally a DNA-binding protein, it is shed from necrotic cells. HMGB1 is able to form complexes with TLR ligands and activate receptor for advanced glycation end products as well as TLR2 and TLR4 (51). The endogenous TREM-1 ligands CD177, PGLYRP1, HMGB1 share common threads in immune cell signaling. Targeting these ligands with molecules that prevent specific ligand interactions with TREM-1 may prove to be of therapeutic use, contingent upon more focused pre-clinical testing.

4.6 Targeting the TREM-1 transmembrane domain

Because TREM-1 is a surface receptor, the creation of synthetic TREM-1 modulators has concentrated on its accessible ligand-binding domain. Alternatively, Sigalov and colleagues approached the task of inhibiting TREM-1 function by targeting its transmembrane domain. According to the signaling chain homo-oligomerization (SCHOOL) model, signal transduction through multi-chain immune recognition receptors is driven by homo-oligomerization of receptor and adaptor subunits (52, 53). In this scheme, ligand binding induces receptor re-orientation and clustering of signaling oligomers that leads to recruitment of effectors, such as tyrosine kinase recruitment to ITAM-bearing adaptors (52, 53). Thus, it is possible to design peptides or other substances that interfere with putative homo-interactions between TREM-1/TREM-1 homodimers or TREM-1/DAP12 complexes.

Using the SCHOOL model approach, a peptide sequence referred to as GF9 was selected from a highly conserved transmembrane core region of human and mouse TREM-1 (see Table 1) (28, 53). The GF9 peptide was synthesized totally of L-amino acids. As predicted, *in vitro* incubation of GF9 peptide with J774 murine macrophages attenuated LPS-mediated induction of IL-1 β , IL-6 and TNF- α compared to control peptide (28). Accordingly, *in vivo* administration of GF9 peptide in mice one hour prior to high-dose LPS challenge significantly improved survival (28). Furthermore, GF9 peptide administration reduced tumor growth in a xenograft mouse model of non-small cell lung cancer (28).

In summary, SCHOOL model-based peptides provide a ligand-independent alternative to the many other peptides derived from the TREM-1 ligand-binding domain. Immunochemical-based and fluorescence resonance energy transfer-based assays will be useful in the screening of related peptides or small molecules that may inhibit TREM-1/DAP12 transmembrane interaction (53).

5. TREM-2 functions and therapeutic applications

5.1 TREM-2 mutations in humans

TREM-2 is primarily expressed on macrophages, monocyte-derived dendritic cells, mast cells, osteoclasts and microglial cells (12, 23). In contrast to TREM-1, the TREM-2/DAP12 complex has shown the power to suppress production of prototypical pro-inflammatory cytokines and to promote phagocytosis in macrophages (6, 12, 54). Also, stimulation of TREM-2 via cross-linking with an anti-TREM-2 antibody enhanced osteoclast differentiation of bone marrow-derived monocyte/macrophages and RAW264.7 cells *in vitro* (55). In humans, rare mutations in TREM-2 or its adaptor protein DAP12 are known to cause the same disorder called polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL; also known as Nasu-Hakola disease) (56). PLOSL is characterized by demyelination of the central nervous system, progressive dementia and bone cysts (56). Features of PLOSL are recapitulated in DAP12-deficient mice, which exhibit osteopetrosis and thalamic hypomyelination (57). TREM-2 has been shown to interact with plexin-A1 receptor, and *plexin-A1*^{-/-} mice display deficiencies in immunity and bone homeostasis (58, 59). However, patients with PLOSL apparently do not suffer severe immune deficiencies (56).

Recently, genome-wide association studies revealed that heterozygous missense mutations in TREM-2 are risk variants for Alzheimer's disease (60, 61). The single nucleotide polymorphism rs75932628 (T allele) results in the substitution of arginine to histidine at position 47 (R47H) of the TREM-2 protein. The R47H mutation is postulated to disrupt the binding of ligands to TREM-2 (61, 62). It will be possible to better determine Alzheimer's disease susceptibility as developing patient databases begin to incorporate TREM-2 polymorphisms (62). Ultimately, because individuals who have heterozygous mutations in TREM-2 still retain one functional wild-type receptor, TREM-2 agonists could be utilized in an attempt to restore TREM-2 function to normal levels.

5.2 TREM-2 decoy receptors

Soluble TREM-2 (sTREM-2) interferes with activation of membrane-bound TREM-2, analogous to the relationship between sTREM-1 and surface TREM-1 (63). *In vivo* administration of recombinant murine TREM-2 in mice worsened their survival in the cecal ligation and puncture model of polymicrobial sepsis, owing to impaired bacterial clearance (63). More specifically, surface TREM-2 can be antagonized by soluble portions of the extracellular domain spanning human TREM-2 (amino acids 13 to 167) or murine TREM-2 (amino acids 19 to 171, or 18 to 164) (2, 64). Through microarray analysis, Tojo and colleagues revealed that TREM-2 expression is highly up-regulated in epididymis adipose tissue from KKA^y mice, a model of obesity and type II diabetes, compared to control wild-type mice (64). *In vivo* administration of recombinant murine sTREM-2 in KKA^y mice reduced blood levels of glucose, insulin, triglycerides and non-esterified fatty acids (64). These results implicate TREM-2 in the etiology of type II diabetes and obesity and warrant further investigation (65).

5.3 2'-O-(2-methoxyethyl)-modified nucleotides as TREM-2 ligands—In attempts to identify TREM-2 ligands, a TREM-2-Fc fusion protein containing its extracellular domain was demonstrated to bind to macrophages and bone marrow-derived dendritic cells as well as various Gram-negative and Gram-positive bacteria (66–68). The results support the notion that natural ligands of TREM-2 may be present on the surface of these cells.

Senn and colleagues demonstrated that certain oligonucleotides containing 2'-O-(2-methoxyethyl), or 2'-MOE, modifications are specific ligands of TREM-2 (69). This is important because oligonucleotides intended for antisense applications have been designed to contain the 2'-MOE modification and to lack CpG and 5'-TC motifs, which activate TLR9. Nonetheless, such oligonucleotides still elicited a pro-inflammatory response in TLR9-deficient mice (69). The stimulatory effect of the 2'-MOE oligonucleotides on cytokine production was TREM-2-dependent, as demonstrated by TREM-2 over-expression or siRNA-mediated knockdown of TREM-2 in RAW 264.7 cells *in vitro* (69).

5.4 Heat shock protein 60 as a TREM-2 ligand

Stefano and colleagues discovered that TREM-2-Fc fusion protein bound to Heat shock protein 60 (Hsp60) on the surface of N2a neuroblastoma cells and astrocytes in culture (70). They also showed that Hsp60 stimulated phagocytosis in microglial N9 cells, and this effect was attenuated by siRNA-mediated knockdown of TREM-2 (70). The role of TREM-2 in phagocytosis is puzzling. Bone marrow-derived macrophages isolated from *TREM-2*^{-/-} mice displayed reduced phagocytosis of *E. coli* and *S. pneumonia* compared to wild-type macrophages (11, 54). In contrast, alveolar macrophages isolated from *TREM-2*^{-/-} mice actually exhibited enhanced phagocytosis of *E. coli* and *S. pneumonia* associated with an increased level of complement component 1q (11). This may explain why TREM-2-deficient mice had a better survival rate than control wild-type mice following infection with *S. pneumoniae* (11).

6. TREM-3, TREM-4 and pDC-TREM

6.1 TREM-3 and TREM-4 functions

Murine TREM-3 is an activating receptor that partners with DAP12, similar to TREM-1 (71). However, the human *TREM-3* ortholog is a pseudogene. Human TREM-4 (also known as CLM-9, CD300Lg, Nepmucin), is a transmembrane glycoprotein found in capillary endothelium, cardiac tissues and in the testis (7, 72). Based on its expression pattern, it has been proposed that modulation of TREM-4 could be therapeutic in vascular and heart disease or as a male contraceptive (72). In mice, endothelial TREM-4 has been shown to mediate lymphocyte adhesion through its immunoglobulin domain (73).

6.2 pDC-TREM functions

A TREM family member referred to as pDC-TREM was found to be selectively expressed on murine plasmacytoid dendritic cells (74). Like TREM-2, pDC-TREM was shown to interact with plexin-A1 receptor. Interference with pDC-TREM/DAP12 signaling with an anti-pDC-TREM antibody suppressed plasmacytoid dendritic cell production of IFN- α

expression (74). These results suggest that pDC-TREM could have an important role in anti-viral responses.

7. TLT receptor functions and therapeutic applications

7.1 TLT-1 functions

TLT-1 and TLT-2, unlike TREM-1 and TREM-2, do not associate with the adaptor protein DAP12 (see Figure 2) (3). Instead, the TLT-1 cytoplasmic tail harbors two immunoreceptor tyrosine-based inhibitory motifs (ITIM) (14, 75). TLT-1 can also be modified by palmitoylation at cysteine 196 within its cytoplasmic domain (76). TLT-1 is expressed in alpha-granules of megakaryocytes and platelets (77). Upon activation, TLT-1 transports to the membrane of platelets where it can enhance Ca^{2+} influx and promote platelet aggregation (15, 78). TLT-1 can bind specifically to fibrinogen (16). A soluble form of TLT-1 (sTLT-1) is also released upon platelet activation and has also been shown to bind fibrinogen and enhance platelet aggregation *in vitro* (16, 77).

Modulation of TLT-1 with sTLT-1 peptides or anti-TLT-1 antibodies could conceivably be useful in treatments for sepsis, clotting disorders and vascular occlusion or thrombosis as in stroke and myocardial infarction (79–81). Altered levels of TLT-1 or sTLT-1 in a patient's biological fluid sample compared to that of a healthy subject could be an indicator of the disease severity (82). Hilden and colleagues proposed a method to exploit TLT-1 as a protein that is restricted to platelets. The covalent attachment of another coagulation factor to an antibody targeted against TLT-1 is a means to deliver the factor directly to platelets (83).

7.2 sTLT-1 as a decoy receptor for TREM-1

Like sTREM-1 and TREM-1-derived peptides, sTLT-1 and TLT-1-derived peptides can act as decoys that bind and trap TREM-1 ligands (16). Gibot and colleagues selected conserved sequences within the extracellular domain of TLT-1, which they referred to as LR17 and LR12 peptides (see Table 1) (16, 84, 85). Recombinant human TLT-1, TREM-1, and LR17 could each be detected by immunoblotting with agonistic mouse anti-TREM-1 monoclonal antibody, used in this case as a "TREM-1 ligand" (84). LR17-scrambled peptide, however, was not detectable by the anti-TREM-1 antibody (84). In a competition assay, LR17 competed away binding of TREM-1 ligand(s) from LPS-stimulated neutrophils supernatant to recombinant sTREM-1 coated on a sensor chip, as determined by surface plasmon resonance (16, 84). The sequences of LR17 peptide from TREM-1 and LR17 peptide from TLT-1 are highly similar (see Table 1). At the same time, the existence of TLT-1-selective ligands other than fibrinogen remains poorly defined.

In vivo administration of human LR12 peptide was shown to improve hemodynamics and protect against hypotension in LPS-challenged Cynomolgus monkeys and in a pig model of fecal peritonitis (86, 87). The investigators suggest that LR12 also protects against endothelial dysfunction, perhaps due to direct actions of TREM-1 expressed in vascular endothelial cells (86, 87). Further studies using peptide blockers of TREM-1 function are warranted to determine if their acute effects on cardiovascular homeostasis might extrapolate to chronic diseases such as hypertension and atherosclerosis (65).

There are many examples of ITIM-bearing receptors that negatively regulate signaling mediated by homologous ITAM-bearing receptors (88). In the case of TLT-1, its ITIM-mediated signaling simply cannot regulate TREM/DAP12 units because they do not share the same expression pattern. Expression of an alternate transcript of TLT-1 called TLT-1s has been found on bone marrow-derived macrophages (89). TLT-1s was shown to interact with TREM-2 via immunoprecipitation, and exogenous over-expression of TLT-1s inhibited osteoclast differentiation of bone marrow-derived macrophages. Therefore, TLT-1s ITIM-mediated signaling could be a negative regulator of TREM-2 function (89).

7.3 TLT-2 functions

The TLT-2 receptor does not associate with DAP12 like TREM-1 and TREM-2, nor does it bear an ITIM like TLT-1. The cytoplasmic tail of human TLT-2 does contain potential tyrosine-based motifs (e.g. YxxV and YxxC) as well as a potential Src homology 3-binding motif (3, 90). TLT-2 expression has been found in the basal or activated state across B cells, T cells, monocytes/macrophages and neutrophils (91). Murine B7-H3 (CD276) was shown to bind to TLT-2. B7-H3-mediated induction of IL-2 and IFN- γ production by T cells *in vitro* was TLT-2-dependent (91, 92). TLT-2 is also reported to bind phosphatidylserine, a phospholipid that can become exposed on the surface of cells as a recognition signal for apoptosis (93). Indeed, the binding of a labeled polypeptide comprising the TLT-2 extracellular domain was enriched on the surface of apoptotic thymocytes as opposed to viable thymocytes. Exogenous over-expression of TLT-2 in NIH/3T3 cells enhanced the phagocytosis of apoptotic thymocytes *in vitro* (93).

Justement and colleagues demonstrated that *in vivo* administration of an anti-TLT-2 monoclonal antibody in mice increased the recruitment of neutrophils to lung and peritoneum (94). Anti-TLT-2 antibody treatment also increased levels of cytokines and chemokines (e.g. IL-6, G-CSF, CXCL1 and CXCL2) in lung tissue of mice *in vivo* and in cultures of primary resident peritoneal macrophages *ex vivo*. Furthermore, TLT-2 ligation with anti-TLT-2 antibody enhanced the production of reactive oxygen species in murine neutrophils stimulated with formyl-Met-Leu-Phe (94). Together, these results establish TLT-2 as a target for modulation of leukocyte activation and chemotactic response.

8. Conclusions

It is notable that the majority of available TREM-1 modulators are simply conserved peptides derived from the TREM-1 or TLT-1 ligand-binding domain, which behave as decoys for endogenous TREM-1 ligands. Despite their similarities, TREMs and TLTs do not exclusively share the same ligand binding properties. Fc chimera proteins of mouse TLT-1, TLT-2 and TREM-2 were found to bind with low affinity to lipids including phosphatidic acid, cardiolipin, and lipid A, whereas TREM-1 and TREM-3 did not (95). In contrast to the targeting of ligand interactions within the TREM-1 extracellular domain, the transmembrane domain is also a target of blocking peptides based on the SCHOOL model (52). A conserved fragment of the TREM-1 transmembrane domain, referred to as GF9 peptide, is presumed to prevent TREM-1/DAP12 interactions by binding directly to the adaptor DAP12 (28, 53). This approach is in direct opposition to the “soaking up” of ligands by TREM-1 ligand-binding domain decoy peptides, which could have unintended off-target effects.

The possibility that TREM-1 and TREM-2 are dynamically regulated at the protein level (i.e. phosphorylation) is hindered by the fact that their cytoplasmic tails lack signaling motifs. However, TREM-1 and TREM-2 are subject to N-glycosylation. TREM-1 and TREM-2 can become reduced from >30 kDa to ~26 kDa following N-deglycosylation, as demonstrated by immunoprecipitation and Western blot (1, 96). This aspect is understudied, and glycosylation of TREMs could play important roles in ligand binding and/or receptor oligomerization.

8. Expert opinion

Therapeutic applications based on TREM family receptors mainly revolve around antagonizing TREM-1 function. The scope of clinical trials based on TREM family receptors is limited. To date, clinical trials related to TREMs have been observational studies designed to test TREM-1 and/or sTREM-1 protein as a biomarker in samples taken from patients with acute, but not chronic, inflammatory conditions (see Table 2). Immunoassays have been utilized in these trials to detect and measure protein levels of TREM-1 and sTREM-1 for prognostic and/or diagnostic value. Other TREMs have yet to be tested as biomarkers. Interventional studies using agents that directly modulate the function of TREM family receptors have yet to be conducted in human patients. The ability of TREM-1 to amplify TLR4 and TLR2 signaling upon co-stimulation has been shown in neutrophils and monocytes, and TREM-1 expression is positively regulated by various TLR ligands (17,18). Therefore, the effects of clinically relevant antagonists of TLR4 and TLR2 could be mediated in part due to inhibition or down-regulation of TREM-1. To this point, clinical trials based on TLR antagonists that have TREM-1 as a primary outcome measure are lacking.

In a genetic clinical study, DNA sequencing was performed on the TREM-1 gene exonic sequence using blood samples from patients admitted for sepsis and from healthy control individuals (97). Blood serum sTREM-1 concentrations were significantly higher in the non-survivor group compared to the survivor group based on 28-day mortality (97). The TREM-1 rs2234237 single nucleotide polymorphism was associated with a significant increase in mortality in patients with sepsis, without affecting serum sTREM-1 level (97). The rs2234237 polymorphism results in a S25T variation in TREM-1 protein. More studies are needed to determine how this and other substitutions in TREM-1 affect its function.

The development of TREM-1 blocking peptides has been based on highly conserved sequences of TREM-1 and TLT-1, giving us greater confidence that pre-clinical research in animals will translate to humans. The first clinical treatment based on pharmacological modulation of TREM-1 will most likely be related to combating sepsis, a major cause of human mortality. Using Cynomolgus monkeys, continuous intravenous infusion with LR12 TREM-1 blocking peptide, subsequent to an intravenous bolus of LPS, prevented hypotension and leukopenia (87). LR12 treatment also reduced the plasma levels of IL-8, TNF- α and MCP-1 compared the control group that received LPS and a scrambled peptide (87). This nonhuman primate study provides a benchmark for future phase I clinical trials to test TREM-1 blocking peptides in humans. Clinical testing of antagonistic anti-TREM-1

antibodies is likewise warranted because the antibodies bind directly to TREM-1 whereas the blocking peptides might only bind to TREM-1 ligands.

As opposed to TREM-1 inhibition, there is also a demand for agonists that stimulate TREM-1 or TREM-2 function. As prime example, activating ligands of TREM-2 may prove to be highly beneficial in the treatment of Alzheimer's and other neurodegenerative diseases (61). Under certain conditions, TREM-1 agonists could be used to enhance the innate immune response. That being said, TREM family members have diverse functions and expression patterns in and out of the immune system. Such heterogeneity lends to the potential that systemic treatment even with specific activators or inhibitors of TREMs could have serious side effects. Recently, efforts have been made to develop cell type-specific delivery systems. For instance, the packaging of TREM-1 blocking peptides to synthetic high density lipoprotein nanoparticles targets their delivery to macrophages and even improves the peptide half-life (28). Strategies for targeted delivery of peptides and small molecules represent a giant leap towards improving the safety of their pharmaceutical purposes.

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• or of considerable interest

•• to readers.

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Article Highlights

- The TREM family of innate immune receptors has a pivotal role in the host-defense system and potential roles in chronic diseases including atherosclerosis, allergy and asthma.
- Pharmacological modulation of TREM family receptors could prove to have therapeutic applications in acute and chronic inflammatory conditions.
- TREM-2 has additional functions in microglial cells and osteoclasts, and certain TREM-2 mutations are implicated to have a causative role in Alzheimer's disease and Nasu-Hakola disease (PLOS).
- Soluble forms of TREM-1 and TLT-1 appear to act as endogenous decoy receptors and are also useful as biomarker for the chronicity and/or severity of several inflammatory conditions.
- Short peptide derivatives of the TREM-1 or TLT-1 extracellular domain are specific blockers of surface TREM-1 activation that compete with ligand binding.
- Critical analyses of the putative ligands for TREMs and TLTs will aid in approaches to develop more specific agonists or antagonists of these receptors.

Figure 1.

Predicted structural domains, motifs and modifications within human TREM-1, TREM-2, TLT-1 and TLT-2 proteins.

Protein depictions were assembled based on amino acid sequence annotations from UniProt and Worldwide Protein Data Bank. Abbreviations: Signal = signal peptide; S-S = disulfide bonds between cysteine residues; Ig-like-V-type = immunoglobulin-like variable-type; TM = transmembrane; Cyt = cytoplasmic; ITIM = immunoreceptor tyrosine-based inhibitory motif.

Figure 2.

Interactions among putative ligands and inhibitors of TREM and TLT signaling.

Notes: DAP12 forms a disulfide-bonded homodimer but is simply represented as a monomer here. The possibility that certain TREM-1 ligands may cross-react with surface TLT-1 and vice versa has not been precluded. Abbreviations: ITAM = immunoreceptor tyrosine-based activation motif; ITIM = immunoreceptor tyrosine-based inhibitory motif.

Table 1

Short blocking peptides derived from conserved sequences within TREM-1 and TLT-1 extracellular and transmembrane domains.

Name	Origin	Domain	Amino acid sequence	Refs.
h-P1	hTREM-1 ^{67–89}	EC-CDR2	LACTERPSKNSHPVQVGRILED	(39, 41)
m-P1	mTREM-1 ^{67–89}	EC-CDR2	LVVTQRPFTRPSEVHMGKFTLKH	(39)
h-LP17	hTREM-1 ^{103–119}	EC-CDR3	LQVEDSGLYQCVIYQPP	(39)
m-LP17	mTREM-1 ^{103–119}	EC-CDR3	LQVTD SGLYRCVIYHPP	(39, 40)
h-GF9	hTREM-1 ^{213–221}	TM	GFLSKSLVF	(28, 53)
m-GF9	mTREM-1 ^{210–218}	TM	GLLSKSLVF	(28, 53)
h-LR17	hTLT-1 ^{94–110}	EC	LQEEDAGEYGCMVDGAR	(16, 84)
m-LR17	m-TLT-1 ^{95–111}	EC	LQEEDTGEYGC VVEGAA	(16)
h-LR12	hTLT-1 ^{94–105}	EC	LQEEDAGEYGCM	(84, 86)
m-LR12	m-TLT-1 ^{95–106}	EC	LQEEDTGEYGCV	(16)

Abbreviations: h = human; m = mouse; EC = extracellular; CDR = complementarity determining region; TM = transmembrane.

Table 2

TREM-1 in clinical trials. List of observational clinical trials using TREM-1 and/or sTREM-1 protein as a diagnostic/prognostic biomarker in samples from patients admitted to intensive care units for various infections and inflammatory conditions.

Condition/disorder	Sample type [ClinicalTrials.gov Identifier]
Sepsis, septic shock, or systemic inflammatory response syndrome (SIRS)	Blood serum [NCT01410578, NCT01388725, NCT02198950, NCT01903668, NCT01333657] Blood (DNA; <i>TREM-1</i> gene exonic sequence genotyped for single nucleotide polymorphisms) [NCT01490424] Sample of infection site guided by positron emission tomography and intravenous 18F-fluorodeoxyglucose [NCT00791310]
Neutropenic fever (chemotherapy for hematological malignancy)	Blood serum [NCT01034059]
Ventilator-associated pneumonia	Bronchoalveolar lavage fluid and/or blood serum [NCT00976157, NCT00645619, NCT01406951]
Acute respiratory distress syndrome	Blood serum [NCT00557414]
Cystic fibrosis (pulmonary exacerbation)	Blood serum [NCT02188758]
Pleural effusion	Pleural fluid [NCT00166894]
Peritonitis	Dialysate of peritoneal dialysis [NCT00173485]
Acute pancreatitis	Pancreatic fluid (fine needle aspiration guided by ultrasound or computed tomography) [NCT01193413]